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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re application of: Applicants	DeBenedetti, Arrigo, *et al.*	:	Docket No:	101611/507550
Serial No.	09/916,017	:	Group Art Unit:	1635
Filed:	July 26, 2001	:	Examiner:	J. Angell
For:	CANCER GENE THERAPY BASED ON TRANSLATIONAL CONTROL OF A SUICIDE GENE			

Cancelled DM 10/21/03

DECLARATION UNDER 37 CFR 1.132

Box Amendment Fee
The Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This declaration under 37 CFR Sec. 1.132 is supportive of the Amendment and Response filed herewith. I, Arrigo DeBenedetti, declare and say:

1. That I am a citizen of Italy, US permanent resident and that I am one of the co-inventors in the above-referenced patent application; that I have been employed by Louisiana State University Health Sciences Center-Shreveport since 1992, that I have been Associate Professor in the Biochemistry Department since 1998, and I was and still am, engaged in a research program in the field of cancer treatment and particularly genetic therapeutics;

2. That I am familiar with the above-identified patent application Ser. No. 09/916,017, that I have reviewed the January 22, 2003, Office Action in the above captioned case, and that I am familiar with the following references cited by the Examiner: Shimogori *et al.* (BBRC Vol. 223:544-548; 1996); van der Velden et al. 1999, cite in IDS, Table 1, p. 90 Koromilas *et al.* (EMBO 1992, cited in IDS) in view of Li B.D. *et al.* (Cancer 1997, cited in IDS) and further in view of Anderson L.M. *et al.* (Gene Therapy 1999, cited in IDS).

3. That I have analyzed the sequence described in Shimogori et al., using a computer program called M-fold, which analyzes possible structures in RNA using Zucker's minimal energy calculations. That the only stem of possible stability is the 47 nt oligonucleotide marked as hatched boxes in the model on page 820 of the paper listed as "cgggguuuggcgggggcgcuccaugggucaggccagccgggccaccc." That this particular structure is destabilized by some bulges and G-U base pairs. That upon calculation of stability, the 5'UTR described by Shimogori would provide a secondary structure conformation having a stability ΔG of about -22 Kcal/Mol. In addition, that the construct described is only about 56% G/C-rich.

4. That the particular region of ODC described in Shimogori *et al.* is insufficient to confer regulation by the level of eIF4E as shown by Shantz LM, Pegg AE. (Int J Biochem Cell Biol. 1999, 31(1):107-22. Review). That the construct described by Shigomori would not work in intact cells but only in cell-free systems like reticulocyte lysate after *in vitro* transcription. That such sequence does not provide the appropriate level of stability ($\Delta G \geq$ about 50 Kcal/Mol) to selectively regulate translation of the open reading frame.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Further declarant sayeth not.

Arrigo DeBenedetti

5-8-03
Date

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considered
DM 10/31/03

Customer No. 26874
PATENT TRADEMARK OFFICE

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Appl. No.	: 09/916,017	Confirmation No. 8138
Applicants	: DeBenedetti, Arrigo, *et al*	
Filed	: July 26, 2001	
Title	: CANCER GENE THERAPY BASED ON TRANSLATIONAL CONTROL OF A SUICIDE GENE	
TC/A.U.	: 1635	
Examiner	: J. Angell	
	:	
Docket No.	: 101611/507550	
Customer No.	: 26874	

SUPPLEMENTAL DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This declaration under 37 CFR Sec. 1.132 is supportive of the Amendment and Response filed herewith. I, Arrigo DeBenedetti, declare and say:

1. That I am familiar with the above-identified patent application Ser. No. 09/916,017, that I am familiar with the following references: Shimogori *et al.* (BBRC Vol. 223:544-548; 1996) and Kashiwagi *et al.* (BBRC Vol. 178:815-822; 1991).

2. That the plasmid called ODC-7K in the Shigomori paper is derived from a plasmid called pODC188 as described in the Kashiwagi reference (BBRC Vol. 178:815-822; 1991). This plasmid contains an mRNA open reading frame preceded by a 188 nucleotides of the 5'UTR of the ODC mRNA. The structure is the same as discussed in the previous declaration by DeBenedetti and is in fact described on page 820 of the Kashiwagi reference.

3. That the particular region of ODC described by the Shimogori *et al.* reference does not provide the appropriate level of stability ($\Delta G \geq$ about 50 Kcal/Mol) to selectively regulate translation of the open reading frame and is insufficient to confer regulation by the level of eIF4E since the construct would have been translated well in the *absence* of polyamines. The full 5'UTR of the ODC mRNA has over 350 nucleotides in length and is capable of being regulated by eIF4E. Therefore, the 5'UTR of the ODC mRNA is that portion of the mRNA that is capable of forming the proper stability in conformation and is regulatable by eIF4E.

4. That the free energy "ΔG" is the free energy of an ligonucleotide, which is a measurement of an ligonucleotide duplex stability. The strength (ΔG) of the resulting

complexes is measured by thermal denaturation or duplex melting. The ΔG can either be expressed as a negative or a positive number depending upon whether you are looking at the stability as a measurement of free energy stored in the structure (negative) or free energy required to melt the duplex (positive). Energy must be released overall to form a base-paired structure, and a structure's stability is determined by the amount of energy it releases. When free energy stored in the structure is a negative value, then the complex formed is in the thermodynamically stable form. Predicted enthalpy, entropy and free energy of duplex formation -- the enthalpy (ΔH), entropy (ΔS), and free energy (ΔG) -- are thermodynamic state functions, related by the Gibbs equation:

ΔG=ΔH-TΔS (at constant temperature and pressure)

where T is the temperature in degrees K. In practice, the enthalpy and entropy are predicted via a thermodynamic model of duplex formation and used to calculate the free energy and melting temperature.

5. That the predicted free energy of an oligonucleotide that contains self-complementary sequences that can form intramolecular secondary structures is calculated as the most stable intramolecular structure of an oligonucleotide. "Secondary structure" refers to regions of a nucleic acid sequence that, when single stranded, have a tendency to form double-stranded hairpin structures or loops. Nucleic acids can be evaluated for their likely secondary structure by calculating the predicted ΔG of folding of each possible structure that could be formed in a particular strand of nucleic acid. Computer programs exist that can predict the secondary structure of a nucleic acid by calculating its free energy of folding. One example is the MFOLD program.

6. That the ΔG as referred to in the specification and claims is given in absolute energy change value and is evident from the context by one skilled in the art. When expressed as a folded state free energy (a negative number), the more negative the ΔG (i.e., the lower the free energy), the more stable that structure is and the more likely the formation of that double-stranded structure. The stability of a secondary structure is quantified as the amount of free energy released or used by forming base pairs or the input energy required to melt such secondary structure, which in the present case would have to be ≥ 50 Kcal/Mol. It would be obvious to one skilled in the art that the present description describes the required to melt the secondary structure since a structure having a positive free energy requires work to form a configuration and hence would be unstable and not form the required structure. Negative free energies release stored work. When quantified as the amount of free energy released or used by forming base pairs, the more negative the free energy of a structure, the more likely is formation of that structure, because more stored energy is released.

7. That for clarity's sake, the stability of the oligonucleotides of the present invention can be described as "wherein the untranslated sequence further comprises a hairpin secondary structure conformation having a stability measured as folded state free energy of ΔG ≤ about -50 Kcal/Mol" instead of in terms of absolute energy change.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Further declarant sayeth not.

Arrigo DeBenedetti

9-22-03
Date

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